ANTIBACTERIAL ACTIVITY OF *PEDALIUM MUREX* (LINN.) ROOT LEAF EXTRACT ON SELECTED PATHOGENIC BACTERIA

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Abstract

The present investigation an antibacterial activity leaf extract of *Pedalium murex* using various organic solvent like ethanol, acetone, ethyl acetate, chloroform and petroleum ether extracts against ten different human pathogenic bacteria includes both gram positive and gram negative. The ethanol, acetone and chloroform leaf extract showed highest antibacterial activity against all the tested bacteria. The low degree zone of inhibition was presented on ethyl acetate leaf extract and it has no activity observed on petroleum ether leaf extract.

Key words - Antimicrobial activity, Disc diffusion assay, Human pathogens and *Pedalium murex*

INTRODUCTION

The therapeutic plants have been used in many forms over the years to treat, manage or control infections by folklore (**Bano *et al.*, 2014**). The plant based traditional therapeutic systems continue to play an essential role in health care about 80% of the world's inhabitants relying mainly on traditional drugs for their primary health care system (**Owolabi *et al.*, 2007).**In India, the therapeutic plantsand their products have an important therapeutic and antimicrobial aid in various ailments.Today there is widespread interest in drugs derived from plants, which leads to thescreening of several medicinal plants for their potential antimicrobial activity. The antimicrobial activities of plants, fruits and vegetable extracts may reside in a variety of different components, including aldehyde and phenolic compounds **(Hussain *et al.*, 2007).** Production of artificial drugs is high and they produce adverse effects compared to plant derived drugs resistance in human pathogenic such microorganisms (**Abiramasundari *et al.*, 2011)**. The pharmacological industries have produced a number of new antibiotics, in generally bacteria have the genetic ability to transmit and acquire resistance to drugs. The

antimicrobial substances are naturally occurring in plant species and it is thought that their influences on the environment can be used as biological control agents **(Gislene *et al.*, 2000)**. The reported by The **Wealth of India, (1966)** that the medicinal plant of *Pedalium murex* is a small herb distributed in tropical Africa, Ceylon, India and Mexico. *Pedalium murex* is demulcent, diuretic and also found to be useful for the treatment of ailments of urinary systems such as gonorrhea, dysuria, incontinence of urine, etc.., (Chopra *et al*, 1999; Khanuja, 2004) *Pedalium murex* commonly called Bada Gokhru in India belonging to the family Pedaliaceae, is distributed in the coastal areas of southern India (Nadkarani, 1982).

MATERIAL AND METHODS

PLANT MATERIAL

The experimental plant of *Pedalium murex* was collected from Kalrayanhills located at Viluppuram district.

PREPARATION OF PLANT POWDER

The selected healthy plant leaf were spread out and shade dried in the laboratory at room temperature for 5-8 days or until they broke easily by hand. The dried plant leaf were ground to a fine powder by using an electronic blender and the powders were stored in a closed container at room temperature for further uses.

PLANT EXTRACTION

SOLVENT EXTRACTS

Fifty grams of the powdered leaf material was boiled separately with 300 ml of each of the solvents viz. methanol, ethanol, acetone, chloroform and petroleum ether in a soxhlet apparatus for 48 h at different temperatures (depends on the boiling point of the respective solvents). At the end of 48 h each extract was filtered through Whatman No.1 filter paper and filtrates were concentrated at room temperature. The paste like extracts were stored in pre-weighed screw cap bottles and the yield of extracts was calculated based on initial and final weight of the container. These screw cap bottles with the extracts were kept in refrigerator at 4. Each of the extract was individually reconstituted by using minimal amount of the extracting solvent prior to use.

ANTIBACTERIAL ACTIVITY TEST (Disc diffusion method)

DISC PREPARATION

The filter paper discs of uniform size are soaked with the compound (plant extract) usually consisting of absorbent paper. It is most convenient to use Whatman No.1 filter paper for preparing the discs. Dried discs of 6 mm diameter were prepared from Whatman No.1 filter paper and sterilized in an autoclave. These dried discs were used for the test.

TESTED MICROORGANISMS

The antibacterial activity of *Pedalium murex* leaf extracts was investigated against ten bacterial species includes gram-positive bacterial pathogens like *Staphylococcus haemolyticus, Staphylococcus lentus, Staphylococcus aureus,* and *Bacillus cereus.* Gram-negative bacterial pathogens *like Escherichia coli, Serratia marcescens, Enterobacter amnigenous, Klebsiella pneumoniae, Klebsiella oxytoca* and *Brevibacterium paucivorans.* These human pahtogens were purchased from Department of Microbiology, K.AP Viswanatham medical college, Tiruchirappalli, Tamil Nadu

PROCEDURE

Sterile liquid Muller Hinton Agar medium (pH 7.4 ± 2) was poured (10-15 ml) into each sterile petriplates. The growth media also seem to play an important role in the determination of the antibacterial activity. This method was followed by **Baur *et al,* (1966)**. After solidification, 100 µl of suspension containing 108 CFU/ml of each test bacteria was spread over Muller Hinton Agar plates. The sterile filter paper discs (6 mm in diameter) were impregnated with 10µl of the 3 mg/ml extracts (30µg/disc) placed on the inoculated agar. Negative controls were prepared in using the same solvents employed to dissolve the plant extract. Chloramphenicol (30µg/disc) was used as positive reference control to determine the sensitivity of the plant extract on each bacterial species. The inoculated plates were incubated at 37° C for 24 h. this experiment was evaluated by measuring the diameter of the inhibition zones. Each assay was conducted in triplicate.

Statistical analysis

Agar disc diffusion activity was performed in three replicates under strict aseptic conditions to ensure consistency of all conclusions. Data of all experiments were statistically analyzed and expressed as Mean ± Standard Deviation.

Result and discussion

In this present investigation an antimicrobial activity various solvent like ethanol, acetone, ethyl acetate, chloroform and petroleum ether extracts were evaluated by disc diffusion method against ten human pathogenic bacteria includes both gram positive and gram negative. The previous reported that the gram positive bacteria have more susceptible that the gram negative bacteria are response to the plant extract in the present investigation. It is in agreement with the previous reported (Nair *et al*, 2005; Karou*et al*, 2005)

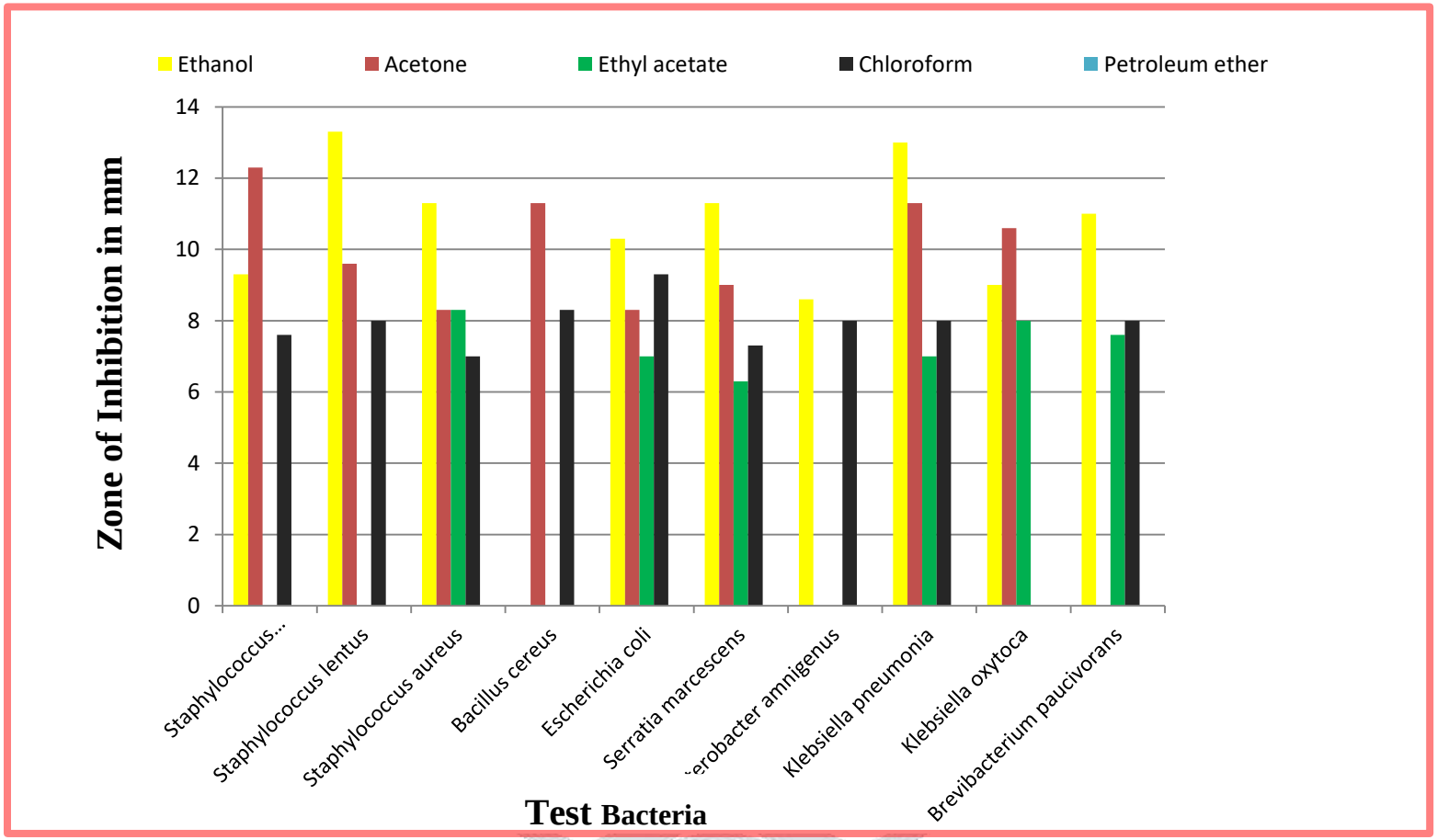
The present investigation results show in Table-1. The ethanol leaf extract exhibited enriched activity against all the tested bacterial pathogens (Figure-2). The maximum zone of inhibition was observed on *Staphylococcus lentus* (13.3±1.5), *Klebseilla pneumoniae* (13±4.5), *Staphylococcus aureus* (11.3±1.1), *Serratia marcescens* (11.3±3.5) and *Brevibacterium paucivorans* (11±4.3). The moderated zones of inhibition against *E. coli* (10.3±0.5), *Staphylococcus haemolyticus* (9.3±0.5) and *klebsiella oxytoca* (9±3.4).the minimal activity was observed against *Entrobacter aminigenus* (8.6±3.5) and it has no zone of inhibition were observed against *Bacillus cereus.*

Table 1: Antibacterial activity of leaf extracts of *Pedalium murex* (Linn) Root on pathogenic bacteria (Disc diffusion method)

Inhibition zone diameter in mm (mean ± SD)											
Test bacteria	**Ethanol**		**Acetone**		**Ethyl acetate**		**Chloroform**		**Petroleum ether**		**Positive Control**
	Experiment (30 µg/disc)	**N**	**Experiment (30 µg/disc)**	**N**	**Experiment (30 µg/disc)**	**N**	**Experiment (30 µg/disc)**	**N**	**Experiment (30 µg/disc)**	**N**	**C (30mcg/disc)**
Gram-positive											
Staphylococcus haemolyticus	9.33±0.5	-	12.3±4	-	-	-	7.6±0.5	-	-	-	28±0
Staphylococcus lentus	13.3±1.5	-	9.6±1.1	-	-	-	8±2	-	-	-	23±0
Staphylococcus aureus	11.3±1.5	-	8.3±2.3	-	8.3±0.5	-	7±1	-	-	-	26.6±0.5
Bacillus cereus	-	-	11.3±1.5	-	-	-	8.3±0.5	-	-	-	21±0
Gram-negative											
Escherichia coli	10.3±0.5	-	8.3±0.5	-	7±1	-	9.3±0.5	-	-	-	25±0
Serratia marcescens	11.3±3.5	-	9±1	-	6.3±0.5	-	7.3±1.1	-	-	-	28±0
Enterobacter amnigenus	8.6±1.5	-	-	-	-	-	8±1	-	-	-	24±1
Klebsiella pneumonia	13±4.5	-	11.3±1.1	-	7±1	-	8±1	-	-	-	12.3±0.5
Klebsiella oxytoca	9±3	-	10.6±5.5	-	8±1	-	-	-	-	-	23.6±0.5
Brevibacterium paucivorans	11±4	-	-	-	7.6±0.5	-	8±1	-	-	-	30±0

'–' represents as 'no inhibition'
N – Negative Control
C – Positive control (Chloramphenicol)

Fig 1: Antibacterial activity of leaf extracts of *Pedalium murex* (Linn) Root on pathogenic bacteria (Disc diffusion method)



The extreme zone of inhibition on acetone leaf extract against *staphylococcus haemolyticus* (12.3±4.0), *Bacillus cereus* (11.3±1.5) and *Klebsiella pneumoniea* (11.3±1.1) the moderated activity was observed against *Klebsiella oxytoca* (10.6±5.5), *Staphylococcus lentus* (9.6±1.1) and *Serratia marcescens* (9±1). The lowest zone of inhibition against *Staphylococcus aureus* (8.3±2.3). No zone of inhibition on acetone leaf extract against *Enterobacter amnigenus* and *Brivibacterium paucivorans*. The extract obtained using ethyl acetate solvent showed the moderated zones of inhibition against *Staphylococcus aureus* (8.3±0.5), *Klebsiella oxytoca* (8±1), *Brivibacterium paucivorans* (7.6±0.5), *E. coli* (7±1) *Klebsiella pneumoniea* (7±1) and *Serratia marcesccens* (6.3±0.5) and it has no activity against *Staphylococcus haemolyticus*, *Staphylococcus lentus*, *Bacillus cereus* and *Enterobacter amnigenus*. The chloroform leaf extract showed moderated zones of inhibition against *E. coli* (9.3±0.5) *Bacillus cereus* (8.3±0.5), *Enterobacter amnigenus* (8±1), *Klebsiella pneumoniea* (8±1), *Brevibacterium paucivorans* (8.3±0.5) and *Staphylococcus lentus* (8±2). The lower activity was observed against *Staphylococcus haemolyticus* (7.6±0.5), *Serratia marcescens* (7.3±1.1) and *Staphylococcus aureus* (7±1) and it has no activity against *Klebsiella oxytoca*. The petroleum ether leaf extract solvent did not showany antibacterial activity. The previous reports on ethyl acetate fraction of *Pedalium murex* flowers possesses antibacterial activity against *Salmonella typhi, Escherichia coli, Enterococcus faecalis, Bacillus cereus, Bacillus substilis* and *Lacto bacillus*.(Prabhakaran *et al*, 2016).

CONCLUSION

It is important to search out and promote medicines that are plant-based. This work will help to identify active ingredients for the treatment of bacterial diseases. Further experimental are needed to assess the effects of the selected plants on other pathogens.

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